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IMPROVEMENTS IN REARING LARVAL PENAEID SHRIMP BY THE GALVESTON LABORATORY METHOD

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ABSTRACT

Improvements in rearing larval penaeid shrimp by the Galveston Laboratory method are presented in this paper. The use of frozen algae and frozen Artemia nauplii as food for larval penaeid shrimp in hatchery systems is discussed. Also presented are culture techniques for the rotifer, Brachionus plicatilis, and descriptions of its use as a shrimp larvae food intermediate to algae and Artemia nauplii. A lengthy discussion concerning problems and their solutions encountered in the Galveston shrimp hatchery is presented.

Contribution Number 80-59G

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INTRODUCTION

Whether it be for research or commercial intent, one of the more crucial links in attempting to produce cultured penaeid shrimp is the hatchery facility. Without the ability to produce large numbers of healthy postlarvae, growout and maturation are useless. In the past 15 years a vast array of equipment and techniques have been tried and developed for rearing larval penaeid shrimp. Chief among these are the systems developed by the National Marine Fisheries Service, Galveston Laboratory, Galveston, Texas, that are now known worldwide as the "Galveston" method.

The penaeid shrimp rearing techniques developed at Galveston have been described by Mock and Murphy (1970), Salser and Mock (1972), and Mock and Neal (1974). These techniques produced survival rates ranging from 65 to 85 percent while maintaining population densities as high as 260 postlarvae per liter. Problems inherent in the use of live Artemia sp. still remained, however, including larval mortalities incurred during the transition from frozen algae to live feed.

Since the first paper by Mock and Murphy (1970), experimentation

at Galveston has shown that similar densities and survival rates can be achieved using frozen rotifers, Branchionus plicatilis, and frozen brine shrimp, Artemia sp., as foodstuffs. These feeding modifications have simplified the rearing process considerably by eliminating the need for simultaneous live cultures and the crises of aborted shrimp hatches due to the failure of live foodstuff.

This paper reiterates methods used at Galveston and discusses the preparation and use of frozen rotifers and frozen brine shrimp for use as foodstuff in the hatchery. Also presented are several other items of interest related to the Galveston culture system. Recently, bakers' yeast has been successfully substituted for frozen algae as foodstuff in the Galveston hatchery; its use is described in another paper (Mock et al., 1980).

MATERIALS AND METHODS

Hatchery

The conical fiberglass hatchery tanks used at Galveston have a capacity of 2000 liters and are fitted with airlift pumps, center screens, and external in-line filters. The airlift pumps keep both the larval shrimp and foodstuff evenly distributed throughout the water column while the water in the tank can either be discharged or recirculated with the center screen in place by operating the drainage or filter housing valves. Detailed descriptions of this equipment have been described by Salser and Mock (1972).

Foodstuff Organisms

Algae culture - The basic techniques for culturing algae at the Galveston Laboratory have been described by Kenslow (1970) and Griffith et al. 1973). A 10 ml innoculum of either Skeletonema costatum or Tetraselmis chuii may be expanded to a 320 liter culture in 16 days. The algal cultures are then concentrated by harvesting through a cream separator and the resulting concentrate is frozen in plastic cups as described by Mock (1971.) S. costatum is frozen in quantities providing densities of 25,000, 50,000, or 100,000 cells/ml while T. chuii is packaged in quantities providing densities of 5,000, 10,000, or 20,000 cells/ml when either is added to the 2000 liter hatchery tank.

Rotifer culture - Rotifers, primarily B. plicatilis, are cultured in rectangular fiberglass tanks (1.x m x 0.6 m x 0.6 m; working volume of 400 liters) under a fiberglass shelter using natural light and ambient temperature as described by Fontaine and Revera (1980). Best growth occurs on sunny days at salinities of 28-32 ppt, temperature of 27-29° C, and pH of 7.7-8.7. The rotifers are fed a water slurry of torulose yeast at a rate of 0.2 g/liter. The culture tanks are stirred each morning after feeding but are not aerated, which allows the tanks to become anaerobic. It appears that anaerobic conditions are essential for rapid proliferation of rotifers. Population densities of 500 to 700 rotifers/ml are reached within 15-20 days. The rotifers will aggregate at the surface 1 to 2 hours after stirring the tanks and those cultures that have attained a density of 500/ml are harvested. Rotifer cultures apparently can be harvested daily for a

period of 45 days, once production density has been achieved, without causing a permanent reduction in the standing crop.

Harvesting is accomplished by skimming the surface of the tank. The rotifer harvest is concentrated by mixing thoroughly with an equal volume of deionized water in a 2 liter separatory funnel. The deionized water shocks the rotifers that then settle to the bottom where they may be removed and frozen. Currently, however, drained rotifers are resuspended in clean seawater (28-34 ppt) and aerated gently for 24 hours. The rotifers are poured through a 69 μfilter, diluted to 1 liter with fresh seawater and fed frozen T. chuii at a level of 1,000,000 cells/ml after microscopic examination has ascertained that the rotifers' digestive tracts have been emptied. The rotifer-alga solution is gently stirred and after 2 hours the rotifers are again harvested by filtering through a 69 μ net. An aliquot count is made and the rotifers are frozen in seawater in plastic cups in quantities yielding levels of 5, 10, and 20/ml when added to the 2000 liter hatchery tanks.

Artemia hatching - An earlier hatchery system for Artemia has been described by Salser and Mock (1972) but decapsulated Artemia cysts, as documented by Bruggeman and Sorgeloos (1979), are also used. Once the Artemia concentration is established with the method of Salser and Mock (1972), a predetermined amount is measured into a container for freezing. The container should be deep enough to allow for 2 to 3 cm of liquid. Convenient densities for use with the 2000 liter tank are 0.5/ml, 1.0/ml, 2.0/ml, and 3.0/ml.

Preparation for feeding - S. costatum and T. chuii may be thawed in either distilled or deionized water. The algae is poured through a fine mesh screen (45 micron) as it begins to thaw. Large particles that are retained are mashed and then washed through the screen. Rotifers must be thawed in seawater to prevent lysing before being passed through the 45 μ screen for clump elimination.

Examination of frozen Artemia containers has shown that freezing induces a physical separation of unhatched cysts and nauplii. The unhatched cysts are stratified in the surface layer. If enough water is initially put in the container before freezing, the surface layer containing the unhatched cysts may be flushed away with tap water after freezing. The frozen block of nauplii can then be either thawed in seawater and poured in the hatchery tank or the frozen block itself may be placed in the hatchery tank where it will thaw in about 5 minutes.

Water quality and treatment - All seawater used for shrimp culture at Galveston is filtered through a 5 μ filter and treated with ethylene-dinitrilo tetra-acetic acid disodium salt (EDTA) at 0.01 g/liter and Erythromycin—[1/] at 5 at 5 mg/liter. Salinities are adjusted to a minimum of 28 ppt with Instant Ocean (a synthetic sea salt) and temperatures are maintained at 28° C. with a thermostatically controlled immersion heater.

Hatchery Procedures

The Galveston hatchery systems have been used successfully to culture a wide variety of crustaceans and finfish (Table I). The most serious fault with the system in regards to crustaceans has been the dependency upon wild gravid females collected by shrimp boats from offshore

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1/ Reference to trade names in this paper does not imply endorsement by the National Marine Fisheries Service, NOAA.

for spawning stock. In August 1979, the Galveston Laboratory successfully matured, mated, and spawned the blue shrimp, P. stylirostris (Brown, et al. 1980.)

Spawning - The female shrimp that appear to be gravid are separated and placed in individual containers. The segregation of females allows for discarding of incomplete spawns, separation of spawns occurring at different times to reduce cannibalism, and provides for a more homogeneous population in the hatchery tanks. After spawning, the female shrimp is removed, the spawning water is replaced with fresh seawater, and the eggs are counted. The eggs remain in the spawning vessel until hatching is complete. A larval population count is then made and the nauplii transferred to the hatchery tanks. The transfer is accomplished by one of two methods: (1) the entire contents of the spawning container is emptied into the hatchery tank, or (2) the nauplii are attracted to the surface by light-attraction and removed by siphoning.

Larvae

Nauplius - The water in the hatchery tank is filtered through a central screen (69 μ) and is recirculated through an external in-line filter (10 μ) during early larval stages. Water filtration continues until the addition of food, at which time it must be discontinued to prevent food loss.

The larval shrimp are absorbing their yolk sac throughout nauplier stages I - V and do not require feeding. Their metamorphosis to protozoea should be anticipated, however, and food (algae) added to the tank during the later nauplier stages. Under the temperature regime described, Nauplius IV

of P. aztecus and P. setiferus usually occur in the afternoon; Nauplius V in the evening; and Protozoea I the following morning. S. costatum is added at a level of 50,000 cells/ml at late N-IV or early N-V (usually in the afternoon) in preparation for the first larvae to metamorphose. Only S. costatum is fed until 75 percent of the population has metamorphosed to the late stage of Protozoea II. T. chuii is introduced at a level of 5,000 cells/ml once this stage is reached. The T. chuii level is increased to 20,000 cells/ml while allowing the remaining S. costatum to be grazed.

Rotifers, B. plicatilis, are added to the tank at a level of 20/ml once the entire population has metamorphosed to Protozoea III. Care must be taken, thereafter, to prevent the rotifer density from dropping below 10/ml.

Mysis - The density of rotifers should be maintained at a level of 20/ml during Mysis I and early Mysis II. Frozen Artemia may be introduced at a level of 1.0/ml at late Mysis II. The Artemia level should be increased to 3.0-4.0/ml when it is evident that the level of rotifers has dropped and that the Artemia are being consumed. At Mysis III, live Artemia are substituted for frozen Artemia at the same level.

Postlarvae - An Artemia level of 3.0-4.0/ml is maintained until the postlarval shrimp are 3 days old (PL-3). If the standard 2000 liter conical tanks are being used the population must be harvested at this time to prevent widespread cannibalism.

Feeding levels - It is of paramount importance to maintain the required density of food in the tanks at all times. The shrimp must never be allowed to graze the food below these specific levels. Therefore, food

density must be increased as food requirements become greater. A suggested feeding regime for all stages of larval development is illustrated in Appendix I (actual experimental data).

DISCUSSION

In the decade following the publication of Cook and Murphy (1969) research efforts at the Galveston Laboratory were guided primarily by the need to solve known problems. During this period of experimentation, each new study evolved its own set of new problems to be overcome. These problems, difficulties and their solutions are discussed here.

Hatchery - Although the conical hatchery tank has proven satisfactory for the culture of larval shrimp at Galveston, it is necessary to harvest the postlarvae after they are 2-3 days old to reduce cannibalism. It woul dbe economically advantageous, however, to rear the postlarval shrimp to a larger size in the hatchery vessel before stocking in ponds or raceways. Semi-closed penaeid shrimp culture raceways, as described by Mock et al. (1973), used as hatchery tanks may be the answer. In Natal, Brazil, Dr. Tupan de Souza, General Coordinator of the Shrimp Culture Project, has built six concrete raceways each with a working volume of 3 cubic meters. They routinely culture 800,000 twenty to twenty-five day old postlarvae (Penaeus brasiliensis) per raceway beginning with a stocking density of 300 per liter or a total of 1,000,000 larvae. It should be pointed out that the raceway as a hatchery tank has not been tried in Galveston, although we plan to do so in the near future.

Regardless of whether a conical tank or a raceway is used as the primary hatchery vessel it is necessary to use airlift pumps. The airlifts

continuously return food from the bottom to the surface resulting in an even food distribution throughout the hatchery tank. An even distribution of foodstuff in the water column is absolutely essential for early larval stages of penaeid shrimp that are primarily pelagic.

Larval shrimp do not have the ability to search for food, but must come in contact with it. Therefore, it is the density (particles per milliliter) throughout the water column that determines if the larvae are going to graze or starve.

Algae - Initially, seawater was used to thaw frozen algae and the algae were added to the hatchery tank without regard to possible effects on water temperature. Under these conditions the algae tended to mass in clumps near the surface. Elimination of this clumping tendency as well as of the worry of undesirable temperature variations was achieved by thawing the algae in distilled water before feeding and adjusting the temperature to within $\pm$ 2.0° C. of the hatchery water.

Rotifers - In the mid-1970's, the demand for Artemia cysts exceeded the supply, resulting in a sharp increase in price. In fact, between 1969 and 1976 the price of a 1,280 g can of Artemia cysts increased from US $19.80 to $70.00. When a search was begun in 1975 for an Artemia substitute, economics was an important consideration; however, it was not the sole factor. A large difference exists between the sizes of food particles of algae and Artemia nauplii as indicated in Table II. There existed a need for a food organism intermediate in size to algae and naupliar Artemia. Such a feed would not only increase survival rates but would also reduce the required amounts of Artemia.

Michel (pers. comm. Michel, Alain, Center Oceanologique de Pacifique, B. P. 7004 Taravoo, Tahiti, Polynesie Francaise) noted that there is usually a portion of the penaid shrimp population that does not survive the transition from algae to Artemia, perhaps as a result of the change in particle size. Ito (1960) suggested that rotifers could be used as food for a variety of marine organisms, and Hudinaga and Kittaka (1966) reported that rotifers were a good intermediate food to bridge the gap between algae and Artemia in penaeid and finfish cultures. They introduced live rotifers, usually along with the culture medium, directly into hatchery tanks.

In our studies at Galveston, once rotifers are concentrated in deionized water they must be put into a salt solution to be frozen. Comparison studies of thawed rotifers that have been frozen in distilled water and in seawater showed that those frozen in distilled water tended to dis- disintegrate while those frozen in seawater did not.

Artemia - There are a number of problems associated with the feeding of live Artemia to larval shrimp. A great deal of time, effort, and equipment must be invested daily to insure that adequate Artemia are available. It is necessary to maintain a costly surplus stock to safeguard against a batch of cysts that are inferior in quality or quantity.

While freshly hatched Artemia can be concentrated at 10,000 to 13,000/ml and stored in refrigerators at 11° C for several days, careful monitoring is still required to prevent spoiling. Some development still takes place although the lower temperature slows down metabolism. The containers must also be provided with an airstone providing bubbles continously and water changes are required every other day. Artemia have been held in volumes of 20 liters successfully for up to 6 days at the Galveston Laboratory.

Live naupliar Artemia compete biologically with the larval shrimp production. They not only consume algae and dissolved oxygen but they also add their waste products to the system. A portion of the live Artemia fed into hatchery tanks are not consumed and survive to adulthood. This greatly complicates hatchery tank conditions as it results in a tri-partite culture including three distinct populations: larval shrimp, Artemia destined to be consumed by the shrimp, and mature Artemia immune to the predation of larval shrimp.

Tetraselmis levels in the hatchery tank required very close monitoring before rotifers were used as an intermediate food between algae and Artemia. Artemia accept and will very readily graze Tetraselmis; later, if Artemia are introduced into a system with high levels of Tetraselmis (20,000 cells/ml) the algae can be very rapidly consumed. In early experiments this phenomenon provided a perplexing problem. The larval shrimp still requiring Tetraselmis had to compete with the Artemia and any increase in the level of Tetraselmis resulted in dramatic increases in the Artemia population and consequently, Tetraselmis consumption. The larger larval shrimp were grazing Artemia at the same time necessitating their presence. Removing the Artemia population from the hatchery tanks is presently impracticable.

Artemia were hatched, concentrated, frozen, and stored before hatchery start-up. The frozen Artemia were then fed at the same rate as the live Artemia and were found to be equally acceptable to the larval shrimp. Originally, Artemia cysts were hatched and then fed directly to the larval shrimp. If the cysts have

been decapsulated, however, the larval shrimp will consume not only the hatched Artemia but the decapsulated cysts as well. Furtherfore, decapsulated cysts will hatch more readily than the non-decapsulated, even within a hatchery tank.

In recent experiments in the Philippines, at the Southeast Asian Fisheries Development Center, Artemia that had been decapsulated and frozen for 24 hours were thawed and fed to larval shrimp of P. monodon. A number of the cysts were consumed and after a 24 hour period the remaining cysts began to hatch (Mock and Sorgeloos, (pers. comm. Dr. Patrick Sorgeloos, State University of Ghent, Laboratory of Biological Research in Environmental Pollution, Ghent, Belgium). Although the decapsulated cysts sink, the action of the airlift pumps keep them suspended in the hatchery tank. Mock and Sorgeloos observed that postlarvae P. monodon feeding upon adult Artemia ate only the head and upper body regions ignoring the digestive and excretory portions. If the adult Artemia were placed in clean water and allowed to discharge the contents of their digestive tracts and excretory systems, however, the shrimp consumed them entirely (Sorgeloos and Mock, pers. comm.).

Biological control - The free-swimming dinoflagellate, Oxyrrhis marina, has been observed in the hatchery water on occasion. In large quantities it may compete with the larval shrimp for Tetraselmis for food although its presence is not directly harmful. Reproduction is by fission and is very rapid when associated with Tetraselmis. Attempts were made to reduce the density of O. marina in the initial bloom by changing water. The dinoflagellate remained in the tank, however, and continued its consumption of the algae. Microscopic examination of individual dinoflagellates showed recent ingestion of 10-15 Tetraselmis cells.

On another occasion when we observed this condition in the hatchery tank the water was not changed but, instead, the feed was switched from Tetraselmis to Skeletonema. The larval shrimp began to graze the Skeletonema once the remaining Tetraselmis was consumed but the dinoflagellate rejected the Skeletonema and died out. When the Tetraselmis was continued the larval shrimp reverted to the larger feed while the dinoflagellate did not reappear. Population counts of the larval shrimp indicated that they had survived. This technique has since been used successfully several times.

The dinoflagellate, O. marina, may also be eliminated from hatchery tanks by recirculating the water through the interscreen to an external in-line UV-light. The external filter is bypassed during this operation. A germicidal intensity (microwatts/cm^2, u Ws/cm^2) of 2537 Angstroms at pumping rates of 68 and 454 liters per hour have been successful in eliminating O. marina as described by Mock, et al.(1980). The UV-light is not detrimental to the algae since the algae are fed dead.

Air stripping - A yellowish-brown foam will begin to accumulate on the surface of the hatchery water after the addition of food and after the larval shrimp begin feeding and feces are observed. This foam is indicative of a buildup of dissolved organics and should not be confused with the foam sometimes present after the addition of algae.

Early attempts to deal with this problem involved replacing 1/3 to 1/2 of the water but more recently another solution has been devised. The water level of the tank is lowered about 15-20 cm below the tops of the airlift pumps or until only bubbles and no water are being discharged. The airlift acts as a protein skimmer and the foam may be removed by simply

lifting it from the tank. The foam should be examined to make certain that the larval shrimp are not being trapped during this procedure. The water level is raised to its original height in the tank after about 30 minutes or until the bubbles are colorless.

Daily hatchery tank inspection - Although the configuration of the hatchery tank and the action of the airlift pumps keep most of the particles suspended in the water column, some deposition does occur. The interface between a particle and the bottom becomes anaerobic and formation of hydrogen sulfide and methane begins once a particle has settled to the bottom of the tank. Since many of the compounds thus formed are toxic this condition must be eliminated. The bottom of the hatchery tank must be inspected daily and brushed lightly when a buildup is noted.

CONCLUSIONS

The techniques for preparing and using frozen foodstuffs promises better efficiency to some operations previously requiring live feeds. Hatchery projects can now be undertaken with an assurance of an abundant, high quality food supply that could never be absolutely guaranteed with simultaneous live culture procedures. Artemia, when used in frozen form, gain a new dimension of versatility. They can now be used without fear of creating an unwanted extra tier in a hatchery tank population. Frozen foodstuffs also provide a new level of efficiency in meeting feeding requirements. Daily food requirements can be met with precision, and loss of a shrimp hatch does not mean the sacrifice of a costly live food culture. Conversely, the loss of a live food culture does not result in a lost hatch. The most economical use of

space and equipment, in those circumstances where they are at a premium, can now be realized by committing scheduled periods once or twice a year for intensive food culturing.

Development of rotifer culture techniques has filled an important gap in the hatchery feeding cycle of penaeid shrimp larvae. Although the economic desirability of an Artemia substitute has become less critical due to the worldwide proliferation of producers and the concomitant advancements in Artemia culture and usage techniques, the use of rotifers as a transitional food solves the problem of abrupt changes in food particle sizes.

Recent developments have served to reiterate the benefits of the airlift pump in crustacean culture. In those instances where the feeding needs of pelagic organisms require the maintenance of food density levels, it is the method of choice. They make possible full utilization of frozen feeds and decapsulated Artemia cysts. In addition, they have some applications to water quality maintenance problems in the hatchery. For instance, the air-stripping of proteins resultant from the buildup of dissolved organics.

The development by the Galveston Laboratory of techniques to mate, mature and spawn the blue shrimp, P. stylirostris (Brown et al., 1980) has been a major breakthrough in respect to hatchery technology. With an abundant year-round supply of nauplii we will now be able to more effectively place our hatchery in a research mode. Although the systems presently being used have worked well for us in the past, we believe there is room for improvement.

Given a dependable supply of shrimp larvae we will now be able to conduct statistically valid, replicated hatchery studies on such items as conical tanks compared with raceways, UV-light treatments, and a variety of other equipment modifications and developments. Additionally, testing of bakers' yeast against frozen algae as larval foodstuff may proceed and further testing of rotifers, nematodes, tardigrades, and lugworms as food for shrimp larvae may be done. Research, particularly that involving hatchery and maturation technology, can now proceed at an accelerated rate so that penaeid shrimp aquaculture can become more cost effective.

TABLE I. Crustaceans Reared to the Postlarval Stage Utilizing the Galveston Technique

Scientific Name		
		Crustaceans
Penaeus aztecus aztecus	-	brown shrimp
P. brasiliensis	-	Brazilian shrimp
P. brevirostris	-	pink shrimp
P. californiensis	-	brown shrimp
P. duorarum duorarum	-	pink shrimp
P. japonicus	-	banded shrimp
P. merguiensis	-	banana prawn
P. monodon	-	sugpo
P. occidentalis	-	blue shrimp
P. schmitti	-	white shrimp
P. setiferus	-	white shrimp
P. stylirostris	-	blue shrimp
P. vannamei	-	blue shrimp
Xiphopenaeus kroyeri	-	sea bob
Sicyonia brevirostris	-	rock shrimp
Macrobrachium rosenbergii	-	river shrimp
		Finfish
		(with aeration modifications)
Scienops ocellatus	-	redfish
Pogonas cromis	-	black drum
Morona saxatilis	-	striped bass

TABLE II. Size Comparison of Various Feed Items

Skeletonema costatum	-	2 - 4 µ diameter
Tetraselmis chuii	-	13 - 15 µ diameter
Brachiones plicatilis	-	50 - 300 µ (total length)
Artemia (San Francisco Brand)		
Egg Decapsulated		200 µ diameter
Non-Decapsulated		230 µ diameter
Instar 1		0.4 mm (total length)
Instar 2		0.5 mm (total length)
Instar 3		0.6 mm (total length)

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APPENDIX I - DAILY LOG OF HATCHERY TANK I

Penaeus setiferus

Nauplii (N), Protozoea (P), Mysis (M)

Postlarvae (Pl), Skeletonema costatum (S)

Tetraselmis chuii (T)

AUG. - 1976				CELLS PER ml		ROTIFERS PER ml		ARTEMIA FROZEN		ARTEMIA LIVE		REMARKS
DAY	HOUR	LARVAL STAGE	LARVAL COUNT	RESIDUAL	FEEDING	RESIDUAL	FEEDING	RESIDUAL	FEEDING	RESIDUAL	FEEDING	
4	0800											Set-up 2,000 liter tank, 28 ppt, 28°C, add EDTA and Erythromycin.
5	2000	NI	400,000									
6	0915	NI-II	380,000									
	2140	NIII-IV	380,000									
7	0745	NIV-V	380,000									
	0930				50,000 S							
	1200	NV-PI		44,000 S	94,000 S							
	1500			70,000 S	170,000 S							
8	0800	PI	380,000	84,000 S								
	0830				184,000 S							
	1345			123,000 S	173,000 S							
	1500	PI		135,000 S	185,000 S							
9	0800	PI-II	376,000	80,000 S	180,000 S							
	1700	PII		120,000 S	170,000 S							
10	0800	PII		80,000 S	180,000 S							
	1200			150,000 S								
	1700	PII		110,000 S	5,000 T							
11	0800	PII-III	350,000	70,000 S								
				2,500 T	12,500 T							
	1200			65,000 S								
				8,000 T	18,000 T							
	1700	PII		10,500 T	20,500 T							
				60,000 S								
12	0800	PII		7,500 T	17,500 T							Brush tank bottom.
				50,000 S								
	1700			12,000 T	22,000 T							
				45,000 S					10			Change 400 l H_2O
13	0800	PIII-MI		13,000 T								
				37,000 S				5	10			
	1700	MI		7,500 T								
				14,000 S				6	26			

AUG. - 1976				CELLS PER ml		ROTIFERS PER ml		ARTEMIA FROZEN		ARTEMIA LIVE		REMARKS
DAY	HOUR	LARVAL STAGE	LARVAL COUNT	RESIDUAL	FEEDING	RESIDUAL	FEEDING	RESIDUAL	FEEDING	RESIDUAL	FEEDING	
14	0800	MI		2,500 T								Brush tank bottom
				32,000 S		12	22					Change 400 l H_2O
	1700	MI-II		2,500 T								
				28,000 S		14	24					
15	0800	MII		2,000 T					0.5			
				22,000 S		12	22	0.2	2.2			
	1700			18,000 S		18	28	1.0	3.0			Change 400 l H_2O
16	0800	MII-III		18,000 S		13	23	1.5	3.5		1.0	
	1700					10	20	1.5	3.5	0.5	3.5	
17	0800	MIII				16		1.5		2.5	3.5	
	1700	MIII, PL				10		1.0		1.5	3.5	
18	0800	PL	320,000					0.5		1.0	3.0	Harvest 80% survival